DEFENSIVE SECRETION OF PROTEINACEOUS GLUES BY *HENIA (CHAETECHELYNE) VESUVIANA* (CHILOPODA GEOPHILOMORPHA)

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Summary: Observations have been conducted on the chemical composition of the defensive glue secreted by *Henia vesuviana*. When analysed by SDS polyacrylamide gel electrophoresis, two protein bands were observed with apparent molecular weights of approximately 130 000 and 12 000. Further biochemical analysis revealed that no single amino acid constituted more than 11% of the weight of the protein. The secretion does not contain cyanide.

The glue is held in a large gland in each segment and is secreted through pores located centrally on the sternites. Each pore is sealed by a hinged cuticular cap which is momentarily withdrawn to allow the glue to escape. The glue hardens within a few seconds of exposure to air and forms a smooth surface. Glue secreted under 2.5% gluteraldehyde, however, forms distinct fibres coated with spherical globules. These may correspond to the high and low molecular weight proteins respectively.

Henia vesuviana is a soil-dwelling geophilomorph centipede which rests in a characteristic posture exposing its ventral surface towards potential attackers. Each segment of the centipede contains a large gland which secretes a sticky fluid in response to attack from predatory arthropods. This glue hardens within a few seconds and is able to immobilise predatory beetles for more than 20 minutes giving the centipede time to escape (Hopkin & Gaywood, 1987).

In this paper, some aspects of the chemical composition of the defensive glue are described. Preliminary observations have also been made by scanning electron microscopy on the structure of the pores through which the glue is secreted.

Materials and methods

Chemical tests

Henia vesuviana was induced to secrete glue by squeezing the body with a pair of forceps. The secretion was placed immediately onto picrate paper and sealed within a plastic centrifuge tube to test for hydrogen cyanide. If cyanide is present, the paper turns from yellow to red-brown.

For gel electrophoresis, samples of glue from three centipedes were placed immediately after secretion into 6M guanidine hydrochloride, 50 mM Tris.HCl pH 7.7 to aid dissolution and dialysed into 100 mM ammonium bicarbonate. Sample solvent was added to give a final concentration of 3% sodium dodecyl sulphate, 600 mM β-mercaptoethanol and 60 mM Tris.HCl pH 6.7. After boiling for two minutes, samples,

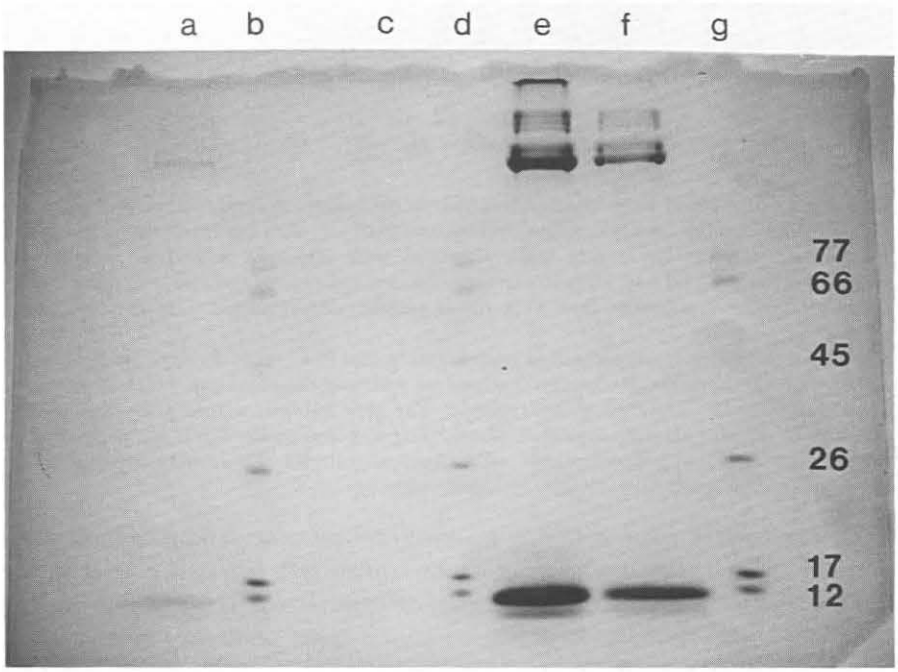


Fig. 1. Polyacrylamide electrophoretic gel of the defensive secretion of *Henia vesuviana*. Tracks b, d and g are molecular weight standards (molecular weights × 10^{-3} are to the right of the gel). Tracks e and f are two different samples of the glue and tracks a and c are 1 in 10 dilutions of the samples loaded in tracks e and f. The glue is composed of two major proteins, one with a molecular weight of about 12 000 and the other of about 130 000. There are other minor higher molecular weight proteins, some of which may be aggregates.

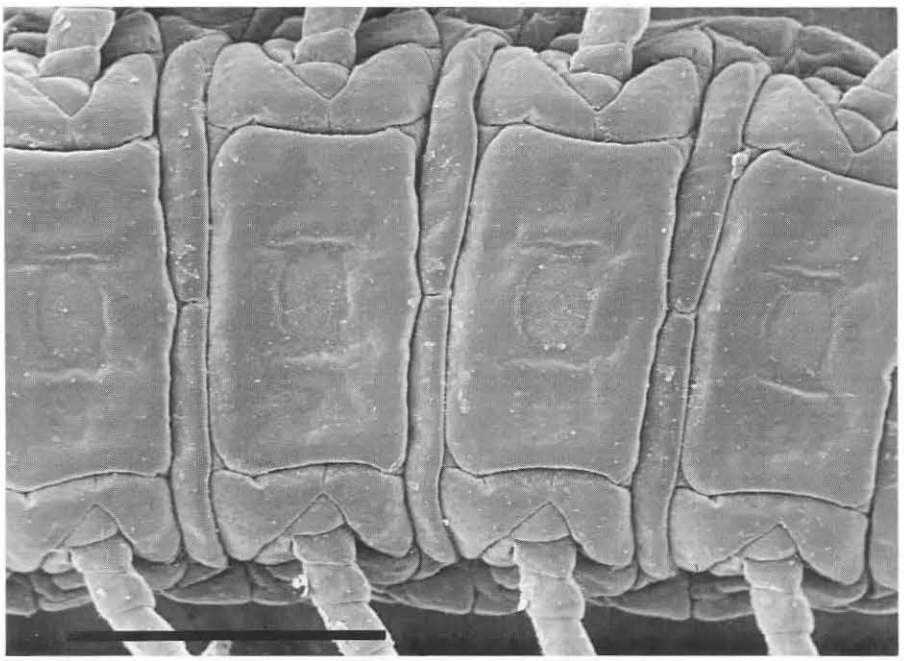


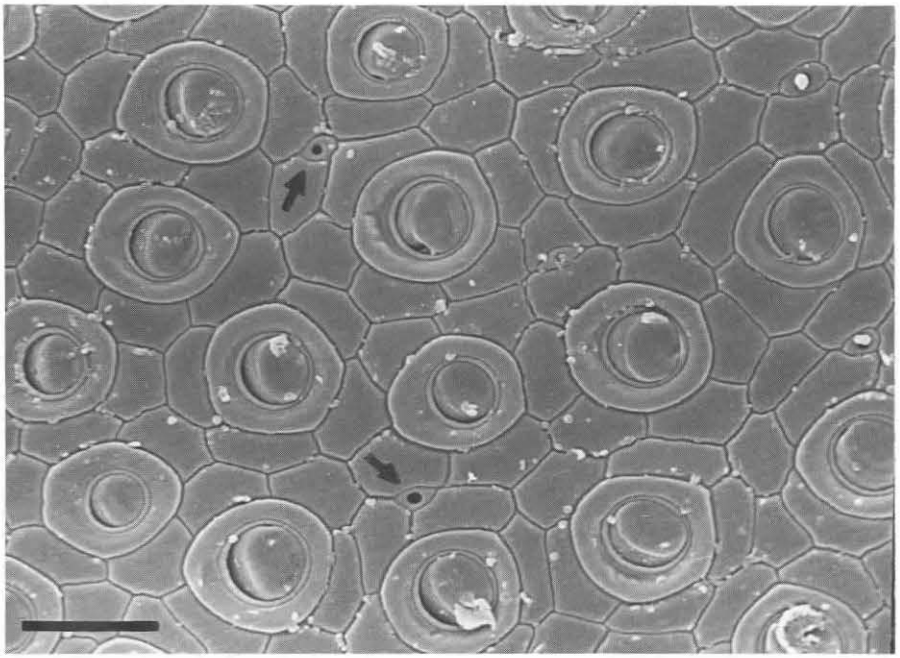
Fig. 2. Scanning electron micrograph of the ventral surface of *Henia vesuviana*. The glue is secreted from pores localised in the central depression on each sternite. Scale bar 1 mm.

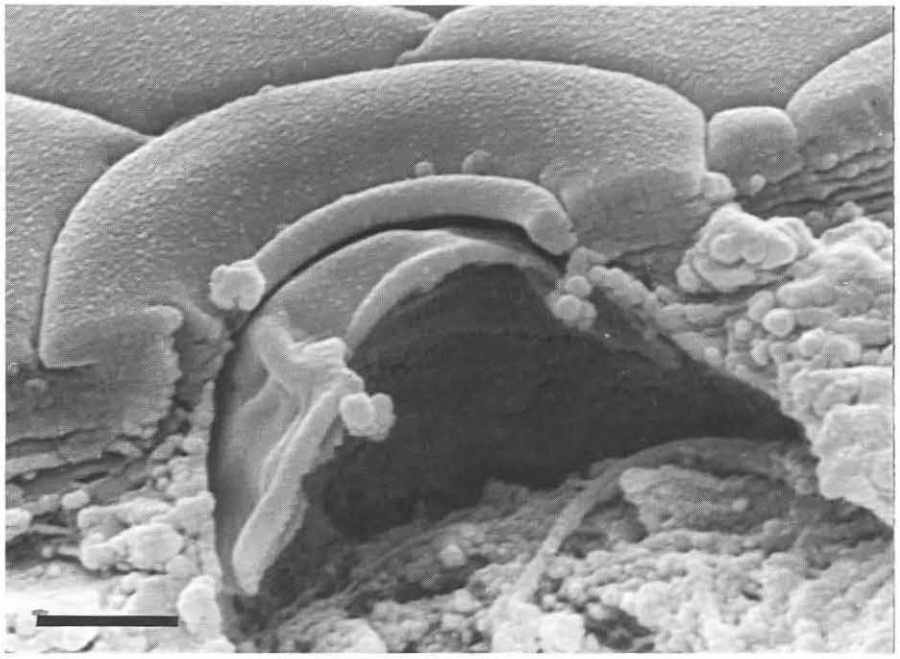
together with molecular weight standards, were loaded onto a 7-15% gradient polyacrylamide, sodium dodecyl sulphate slab gel and subjected to electrophoresis using the discontinuous buffer system described by Laemmli (1970). After electrophoresis, the gel was stained with PAGE blue 83.

For amino acid analysis, the glue was placed in 6M HCl, hydrolysed for 16 hours at 105 °C, and analysed on a Biotronik LC5000 analyser.

Scanning electron microscopy

Specimens of *Henia vesuviana* were placed in 2.5% gluteraldehyde in 0.1 M cacodylate buffer. The centipedes were squeezed with forceps and the glue secreted was stretched into fibres which rapidly polymerised. A longitudinal cut with fine scissors was made through the pore areas of several specimens to reveal their internal structure. The material was critical point dried, mounted on copper stubs, coated with gold in a DC sputter coating unit and examined in a JEOL T300 scanning electron microscope operating at 20kV.





Amino acid	*Moles %*
ASP	9.7
THR	6.3
SER	4.9
GLU	10.2
GLY	8.5
ALA	6.4
CYS	1.8
VAL	8.2
MET	ND
ILE	6.9
LEU	8.2
TYR	0.7
PHE	4.0
HIS	3.0
OH-LYS	ND
LYS	8.1
ARG	5.6
OH-PRO	ND
PRO	7.7

Tab. 1. Amino acid composition of the defensive secretion of *Henia vesuviana* (number of amino acid residues per 100 residues. ND, not detected).

Results and discussion

The defensive secretions of millipedes include chemicals such as cyanide which act to repel an attacker, or other substances which act as sedatives (Eisner et al., 1978). Cyanide has been detected in the defensive secretions of some centipedes (Jones et al., 1976) but in this study, the picrate paper test on the glue of *Henia vesuviana* proved negative. The ability of the glue from *Henia vesuviana* to act as defensive secretion would therefore seem to rely on its capability to physically immobilise an aggressor.

The defensive secretion of *Henia vesuviana* is composed of low (12 000) and high (130 000) molecular weight proteins (fig. 1). It is possible that the fibres which form when the glue is stretched under glutaraldehyde are

Fig. 3. Scanning electron micrograph of pores from which the glue is secreted. Each pore is sealed by an internal cap. Other minute pores (arrows) are interspersed among the glue-secreting pores. Their function is not known. Scale bar 10 μm.

Fig. 4. Scanning electron micrograph of a fracture through a single pore. The pore is sealed by a cuticular cap which is withdrawn to allow the secretion to escape. Scale bar 2 μm.

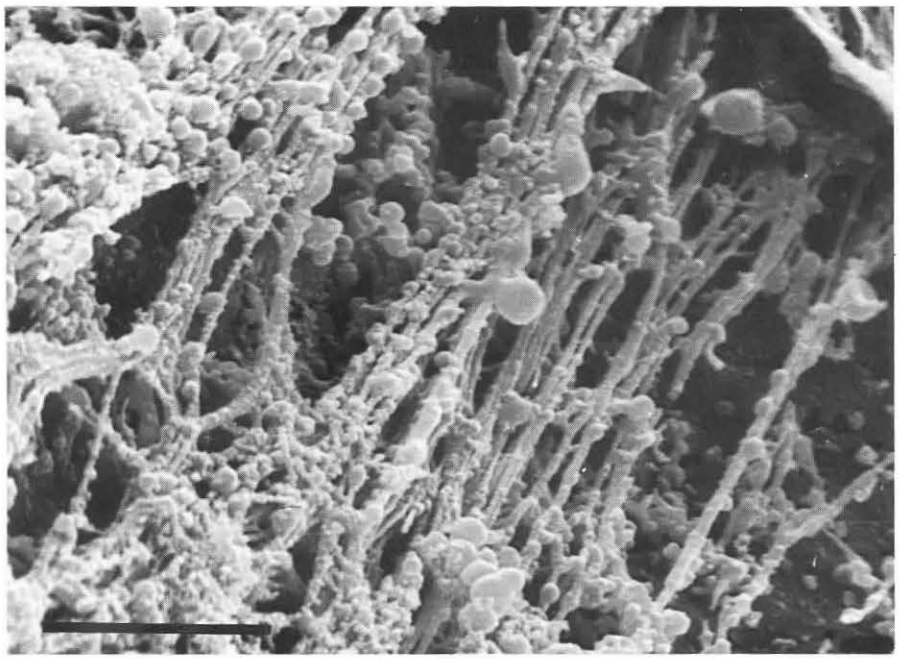


Fig. 5. Scanning electron micrograph of fibres formed from glue secreted and stretched under 2.5% glutaraldehyde. Material coating the fibres has coalesced to form spherical globules. Glue secreted in air does not form fibres. Scale bar 5 μm.

composed of the high molecular weight protein and that the low molecular weight protein forms the spherical globules which are attached to them (fig. 5). When the glue is stretched in air, fibres do not form and the surface is always smooth. The low molecular weight proteins may therefore give the glue surface its stickiness while the high molecular weight proteins can align in the direction of stretch forces to give it its strength. The amino acid composition of the glue (table 1) is quite unlike spider web silk which contains 27% alanine and 20% glycine (Witt et al., 1968).

The central depression on each sternite of *Henia vesuviana* contains about 200 pores through which the glue is secreted (figs. 2, 3). Smaller pores are scattered among these which may secrete a substance onto the cuticle to prevent the glue from sticking to the centipede (fig. 3). Preliminary observations by light microscopy and transmission electron microscopy suggest that each pore is supplied by its 'own' secretory gland and duct. The pore cap (fig. 4) descends into the duct to allow glue to pass through the pore and return to seal the opening within less than 0.5

seconds. In some other geophilomorphs, the pores are sealed by a "plug" of hardened secretion instead of a cuticular cap (Turcato & Minelli, 1989). The ultrastructure of the glue glands within each segment, and the way in which the pore cap is withdrawn, are still under investigation.

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